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Original Research

Enteroviral infections of the central nervous system observed in Bulgaria during five consecutive seasons

Pekova Liliya[1], Nikolaeva Glomb Lyubomira[2], Stoyanova Asya[2], Yordanova Antoaneta[3], Mladenova Zornitsa[2]
[1]Department of Infectious Diseases, Medical Faculty, Trakia University, Stara Zagora,
[2]National Reference Laboratory of Enteroviruses, National Center for Infectious and Parasitic Diseases, Sofia,
[3]Department of Applied Mathematics and Modeling, Faculty of Mathematics and Informatics, Plovdiv University, Plovdiv, Bulgaria

Abstract
Aim: Enteroviruses target various organs and systems of the human body, including the central nervous system (CNS). The aim of the present study was to better understand the clinical features of enteroviral infections of the CNS and the factors influencing the clinical course of disease.Material and methods: A total of 39 patients, aged 3-16 years, admitted to the Clinic of Infectious Diseases, University Hospital in Stara Zagora, Bulgaria, between 2012 - 2017 with confirmed enteroviral neuroinfection were enrolled in the study. Diagnosis was confirmed by laboratory and virological tests. Instrumental investigation was performed if needed. Results: In all cases the disease began suddenly and severely followed by hospitalization within 24 hours. Meningo-radicular syndrome was observed in 25 patients and sustained for average 4,1±1,9 days. Cerebrospinal fluid (CSF) showed pleocytosis in all cases ranging from 57 to 843.106/L, in 9 patients segmented cells predominated at the first lumbar puncture. Mild proteinorachie (0,50 to 0,76 g/L) was found in 17 of the patients. Virological tests revealed echovirus 30 and coxsackievirus B in 24 and 4 patients, respectively. Discussion: All cases in the study fulfill the criteria for a confirmed enteroviral-induced disease. Echovirus 30 was the predominant causative agent, affecting mainly children between 3 and 9 years old. All patients have gone as meningitis. The majority of them had moderate clinical course. Predominance of plymorphonuclear cells in the first lumbar punctate was found in 23% of the patients. All cases had a favorable outcome.

Keywords
Enteroviral meningitis, Echovirus 30, CSF Investigation

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Corresponding Author: Liliya Pekova, Medical Faculty, Trakia University, 11, Armeyska Str.6000 Stara Zagora, , Bulgaria
E-mail: dr.pe_kova@abv.bg GSM: +00359 899 946 450
Corresponding ORCID ID: https://orcid.org/0000-0001-5866-6890

Introduction

Enteroviruses (EVs) are small non-enveloped RNA viruses with icosaedral capside composed of 4 virus-specific structural proteins, of which VP1 elicits type-specific neutralizing antibodies and is primary responsible for serotype designation. Human EVs belong to the genus Enterovirus of the Picornaviridae family. Their original classification included four groups: polioviruses (PV), coxsackie A (CVA), coxsackie B (CVB), echoviruses (E), and unassigned EVs. Improved molecular typing has led to a higher detection rate, and a new nomenclature has been adopted according which human EVs are classified into 4 species - Enterovirus A, B (incl. CVB1-6 and E30), C and D, based on neutralizing assays and sequencing data, mainly of VP1 gene [1].

At present, more than 100 genotypes of human nonpolio enteroviruses (NPEVs) have been identified. As polio eradication approaches, NPEVs are receiving increased attention as significant cause of morbidity and mortality worldwide. NPEVs mainly cause asymptomatic infections, in 80% of the cases, but in symptomatic cases clinical manifestations broadly vary from mild oligosymptomatic conditions such as mild febrile illness, respiratory tract infection, herpangina, gastroenteritis, hand-foot-mouth disease to severe and potentially fatal conditions including aseptic meningitis, encephalitis, acute flaccid paralysis, neonatal sepsis-like illness and myocarditis.

It is estimated that aseptic meningitis caused by NPEVs comprises 48-95% of the cases in which a causative virus is identified [2]. EVs-B are important etiologic agents of aseptic meningitis and encephalitis, especially in childhood. Of them, several echoviruses (E30, E18, E9, E11) and coxsacievirus B (CVB5) have been reported in sporadic cases and outbreaks of aseptic meningitis [3,4]. E30 was the cause of the majority of outbreaks in Europe within the last decade [5].

Aseptic meningitis is a self-limiting illness occurring in all age groups, with neonates most at risk of severe systemic illness. Children are predominantly affected as pediatric EV-associated meningitis is 5-8 times more common than in adults [6,7]. EVs are ubiquitous pathogens that are shed from the gastrointestinal or upper respiratory tract with a seasonal peak in the summer and autumn in temperate climate zones [8].

A representative of NPEVs, namely EV-A71, is known for its neurovirulence for more than 40 years. Interestingly, the first European reports of large and severe epidemics of encephalitis and acute flaccid paralysis due to EV-A71 came from Bulgaria in 1975 [9].

The clinical and virological features of the enteroviral infections that were treated in the Clinic of Infectious Diseases, Stara Zagora University Hospital, Bulgaria, for a period of five consecutive seasons were presented in this study.

Materials and Methods

During the five consecutive seasons, April 2012- August 2017, 39 children with suspected aseptic meningitis were admitted to the Clinic of Infectious Diseases, Stara Zagora University Hospital, Bulgaria. (Figure 1).

All patients were clinically examined at the time of admission, as well as during the hospital stay, at discharge from the hospital and until the third month thereafter. Inclusion clinical criteria for aseptic meningitis were sudden onset, headache, neck stiffness, fever, nausea and/or vomiting, meningeal signs with or without qualitative and quantitative changes of consciousness. The laboratory criteria included inflammatory parameters in the CSF such as elevated albumin, lymphomonuclear cell proliferation, positive albumin-globulin reactions of Pandy and Nonne-Appelt, normal or elevated CSF cell count. All CSF samples were proven negative in bacterial culture. In 3 patients computer tomography (CT) scan of the brain was performed. CSF samples were collected within 12-hours of hospitalization, and were sent for virological examination to the National Reference Laboratory of Enteroviruses, National Center for Infectious and Parasitic Diseases, Bulgaria. All CSF samples were tested by reverse transcription real-time PCR assay (RT-qPCR) using primers targeting the conserved 5'UTR region of the enterovirus genome. All samples were also inoculated in RD and L20B cell lines for viral isolation according to the WHO Polio Laboratory Manual, v4/2004. Microneutralization tests were performed for serological identification of the EV strains isolated using RIVM type-specific antisera pools. In addition, demographics of patients (age, sex) and clinical history (signs and symptoms before hospital admission) as well as clinical examination data were retrieved from the patient's files. SPSS software version 23 was used for the statistical processing of the results obtained.

Results

Patients with EV-confirmed aseptic meningitis were between 3 and 16 years old. (Figure 2).

The sex distribution demonstrated prevalence of males in 29 (61,53%).

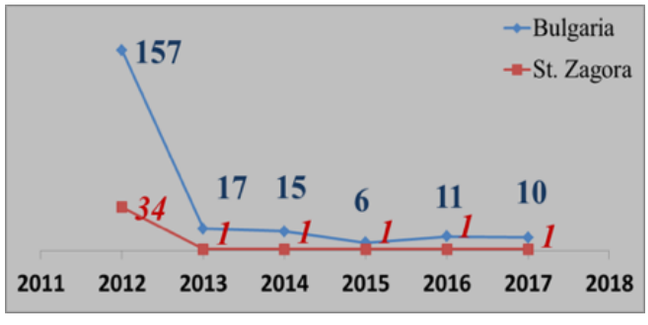

Figure 1. Distribution of aseptic meningitis due to EV in Bulgaria and in Stara Zagora region in particular

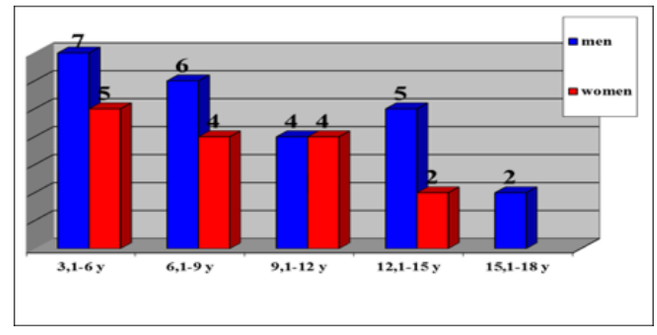

Figure 2. Distribution of our patients with EV meningitis by sex and age

The onset of disease was acute within 1-2 days before hospital admission with high temperature (38,1-39˚C) and severe headache in all cases. Asthenia was observed in 37 (94,87%) of the cases, vomiting in 27 (69,23%) and photophobia in 22 (56,41%). Dyspeptic manifestations such as stomach aches and diarrhea were reported in 11 (28,20%). Fast transient maculo-papular rash was observed in 3 (7,69%) of the patient. (Figure 3).

Meningeal signs were positive at the time of admission in more than a half of patients – 25 (64,1%). Neurological signs such as nuchal stiffness had all of them, Kernig's sign was registered in 25 (64,10%), upper Brudzinski in 22 (56,41%) and lower Brudzinski in 19 (48,71%). All of the patients showed increased tendon reflexes with enlarged reflexogenic zones. (Figure 4).

Duration of meningeal symptoms ranged from 2 to 6 days, mean 4,1±1,9. Two-wave temperature curve with exacerbation of neurological manifestations was noticed in 6 patients (15,38%).

Most of the patients – 26 (66,66%) were with leukocytosis from 16,5 to 24,6.109/L on admission. Other blood and urine tests did not detect pathological anomalies.

Lumbar puncture (LP) was performed in all patients within the first hours of hospitalization. Laboratory tests of CSF indicated inflammation of the meninges. Number of cells ranged from 57 to 843.106/L (mean 438±21,4). Prevalence of the lymphocytes was observed in 30 (76,92%), and in the rest 9 (23%) patients prevailed polynuclear cells.

Elevated albumin in CSF was shown in 17 patients (43,59%) and ranged from 0,50 to 0,76 g/l (0,64±0,2) only for the first LP. The level of CSF sugar was within reference ranges in all patients. Second LP was performed in 19 patients (48,71%), and laboratory parameters showed normal values of albumin and reduced values of cells. Only lymphocytes were found in the sediment.

CT scan performed in 3 (7,69%) of the patients revealed no brain changes.

All admitted patients were epidemiologically investigated.

The etiological diagnosis of EV-induced aseptic meningitis was done in the National Reference Laboratory of Enteroviruses, NCIPD, Sofia, Bulgaria by classical virological methods and molecular-based techniques. All CSF samples were positive by RT-qPCR, while viral isolation on RD cell line was successful for speciments collected from 28 patients. Microneutralization test with RIVM type-specific antisera pools revealed E30 in 24 patients, while in 4 children CV-B was identified.

All patients received glucose-saline solutions to overcome intoxication, as well as anti-edematous agents and corticoids. Systemic antibiotics were applied in 12 (30,76%) of them who presented with severe EV-associated disease with leukocytosis and predominance of neutrophils in the CSF sediment. A total of 9 (23,07%) of the patients were treated with bioproducts such as fresh frozen plasma.

The above therapy led to gradual improvement in all of the patients. One month after discharge, 32 (82,05%) showed complete recovery, while at 7 (17,94%) there was a non-constant headache and a bad appetite. At the third month these complaints disappeared.

Discussion:

In the present study, the clinical and virological features of 39 patients hospitalized due to aseptic meningitis during five consecutive years were assessed. All cases fulfill the criteria for a confirmed EV-induced disease: characteristic changes in CSF biochemical markers, negative results in bacterial culture of CSF, and EV detected as etiologic pathogen by viral isolation, detection of EV RNA or positive serology.

In line with the study of Amarilyo et al. [10], the most common clinical presentation of pediatric EV aseptic meningitis in our study was fever accompanied by vomiting and meningeal symptoms such as headache, neck stiffness, nuchal rigidity, Kernig's and Brudzinski's signs. However, dyspeptic manifestations such as stomach aches and diarrhea were observed in only 28,20% of our children opposite to the results of Nowak et al. who reported in a study of EV meningitis pre-existing gastrointestinal complains in 79% of the cases [11]. In addition, vomiting and photophobia were registered in 69,23% and 56,41% of the patients, respectively. We consider vomiting as a symptom of the meningeal-radicular irritation and not a manifestation of a dyspeptic syndrome. Nearly half of the patients reported myalgia - much more than reported in the literature [12].

The findings of elevated white cell count in CSF coincided with the findings of the majority authors. The mean value of CSF cytosis in our study was 438±21.4.106/L, higher than that observed by Ahlbrecht J et al. who found 115.106/L [13].

Predominance of plymorphonuclear cells in the first lumbar punctate was found in 23% of the patients in contrast with some studies that showed this feature in more than 50 % of cases [14,15]. In 17 (43.59%) of the patients light proteinorachia of 0.76 g/L was found. Although proteinorachia in enteroviral meningitis is a poor prognostic mark according to Mulford et al. [16], our patients had a good diseases outcome.

Aseptic meningitis may be induced by various etiological agents - viral, bacterial or fungial pathogens, drug-related, cancer-associated, autoimmune, etc. In clinical practice, rapid

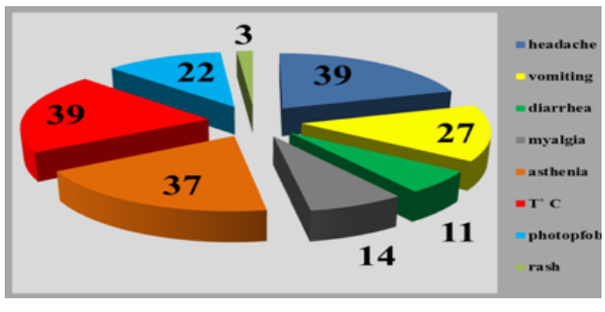

Figure 3. Clinical presentations of the somatic status of our patients with EV meningitis

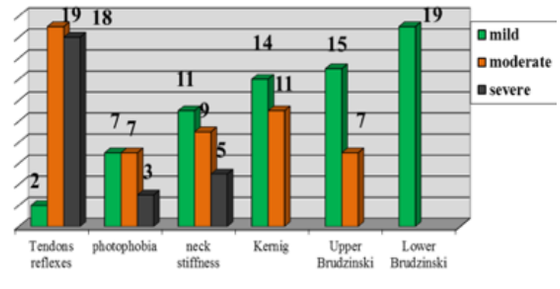

Figure 4. Clinical presentations of the neurological status of our patients with EVmeningitis

and accurate diagnosis of EV-associated disease is of great importance because it has the potential to diminish costs for antibacterial treatment and reduce length of the hospital stay. Virus isolation of EVs in cell culture, which at present is the 'gold' standard diagnostic procedure, is in fact time consuming and has low sensitivity. Nevertheless, it is capable of providing data on the EV serotypes in circulation [17]. While establishing the EV serotype is of negligible interest to the clinicians, it is of great importance from an epidemiological and social point of view. Nowadays, molecular methods for diagnostics such as PCR and sequencing, are becoming more and more accessible. Implementation of those methods in routine laboratory diagnosis of EV infections/diseases will help for obtaining accurate results in a timely manner, that will contribute to a significant reduction of the costs for hospital treatment.

Serotypes of Enterovirus B, i.e. E30 and CV-B, were detected in 28 of the 39 patients with aseptic meningitis – 71,79%. The CSF was the specimen used for virus detection in the study. For EV-induced infections, stool sample was specimen with the highest sensitivity for establishing an acute infection, regardless of clinical presentation [18]. In the other 11 patients, the EV strain was not successfully serotype identified probably due to low viral titres in CSFs and lack of stool samples of those patients.

E30 has been reported as one of the most common EV strain involved in the sporadic and epidemic cases of aseptic meningitis. Outbreaks of E30 were have been registered in many European countries (Greece, Italy, France, etc), as well as in Asia and North America [5, 19, 20, 21, 22, 23].

In Bulgaria, E30-associated outbreak was registered in 2012, and among 157 patients with aseptic meningitis investigated, E30 was detected in 74.5% of them [24]. According to the literature, such epidemics of neuroinfections caused by the same virus have been reported in many places around the world in 2012 [22,25]

Epidemiological data collected among our 34 patients during the outbreak in 2012 revealed close contacts in the kindergarten, at school, or with close relatives of the sick persons.

Since 2012, single sporadic cases have been reported both in the country and in the Stara Zagora region.

Conclusions:

* Enteroviral meningitis may be always suspected in children with the meningeal syndrome, especially when accompanied by dyspeptic manifestations and rash during the summer months.
* Ambiguous data on leukocytosis and predominance of polynuclear cells in the CSF sediment at the first LP requires further clarification.
* Although some patients presented severe clinical course of the disease, the severity of the illness was a moderate one in the majority of the deseased and there was a favorable outcome with complete recovery for all of the patients.
* E30 was the most common cause of EV aseptic meningitis in this study.
* E30 affected most often children aged between 3 and 9 years.
* Fast and reliable EV detection in aseptic meningitis cases may be useful for clinicians in improving current practices of patient care, while EV strain identification is of great importance for monitoring the epidemiology of EV infection and controlling the EV transmission at the population level.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.
(All procedures performed in this study are in accordance with ethical standards of the Ethics Committee at the University Hospital in Stara Zagora, Bulgaria and the Helsinki Declaration of 1964 and its later amendments or comparable ethical standards. All data used in this study is given with the explicit consent of the patients' parents as they are minors. The approval was sought and received.)

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Conflict of interest
None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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